DETECTION AND IDENTIFICATION OF BACTERIA USING INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY

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ABSTRACT

In this study, an inductively coupled plasma mass spectrometer (ICP-MS) was used for the inorganic chemical characterization of biological materials. ICP-MS has the advantage of sub-nanogram/gram detection limits for most elements making it a sensitive tool for the detection and characterization of aerosolized biological material. Microgram-sized samples of Bacillus subtilis spores (BG), Bacillus subtilis vegetative cells (Bg) and Bacillus thuringiensis (Bt) were analyzed and exhibit significant differences in selected elemental ratios (i.e.: Pb/Ba, Mg/Ba, Mn/Ba, P/Ba, Cu/Ba, Sr/Ba, Zn/Ba, V/Ba, and Co/Ba). These results demonstrate a unique signature reflecting the processing history of each organism.

INTRODUCTION

Methods for the detection and characterization of airborne biological materials such as bacteria, using their organic composition are fairly well developed. Unfortunately, success has been limited in developing methods for the detection and identification of biological materials using an inorganic chemical fingerprint. However, in this study the use of inductively coupled plasma mass spectrometer (ICP-MS) for the purpose of chemical characterization of biological materials such as bacteria, fungi and viruses has provided promising results. The use of the ICP-MS technology will initiate improvements in the detection and characterization of airborne organisms including a forensic record of the way in which the organism was cultured and processed. ICP-MS offers several attractive features for trace element studies including rapid multi-element analysis and very low detection limits typically in the pg/g to ng/g range for most elements. Therefore, the purpose of this study is to 1) demonstrate the capability of the

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ICP-MS as a method to detect and identify milligram sized samples of bacteria in suspension, 2) demonstrate the ICP-MS has the potential to act as a highly sensitive detector of aerosolized biological materials and 3) illustrate the differences in chemical signatures as a function of bacteria species, growth history and conditions.

INSTRUMENTATION

A VG PlasmaQuad ExCell ICP-MS was used for these experiments. The instrumental parameters are summarized in table 1.

Table 1. ICP-MS operating conditions

rf power, frequency	1350W, .40 MHz
coolant argon flow	12.70L min^{-1}
auxiliary agron flow	0.70 L min^{-1}
nebulizer agron flow	1 L min^{-1}
sample uptake rate	90s
detector mode	pulse mode
scanning mode	peak hopping
dwell time	10 ms
replicates	3
sweeps	150
channel per mass	3
channel spacing	0.02
acquisition time	58 s
mode	peak jump
internal standard solution	Indium 1ng/ml

MATERIALS AND METHODS

Bacteria samples. Bulk samples of Bacillus subtilis (BG spores), Bacillus subtilis (Bg vegetative cells) and Bacillus thuringiensis (Bt) received from the SBCCOM Edgewood Facilities, Aberdeen Arsenal were used for this experiment.

Internal Standard Solutions. Drift in the sample signal is common over time with the ICP-MS method. As a result, an internal standard is used to monitor and correct for instrumental drift and possible matrix effects during an analysis. In these experiments, all samples, standards and blanks were spiked with a 1 ng/g In. The In detected in each sample through out the run can be compared to the In detected in the first sample (typically a blank) analyzed. A ratio of >1 requires a negative correction where as a ratio of <1 will require a positive adjustment. Drift corrections are typically small and replicate analyses during a run confirm the validity of the correction.

Preparation of bacteria samples. Initially, the samples were digested. First, approximately 100mg of each sample was weighed into a clean 30 ml Teflon vial. Then 5 mL of 30% nitric acid was added and the sample was heated to a constant temperature of 150^{o}C for 3-5 days. After 5 days, the sample was taken to dryness. The dry sample was then re-dissolved using 3 mL of 30 % hydrogen peroxide in order to oxidize any remaining organic matter present. The samples were heated at 150^{o}C for 4-5 hours, and then taken to dryness. Finally, 1 mL of 30% nitric acid and 20 mL of deionized water was added to put the sample back into solution. This method yielded inconsistent recoveries, which led to the development of the suspension method described below.

Suspension of crushed dried bacteria samples more accurately simulates the size range and concentration of spores in an aerosol sample and is therefore more appropriate for the goals of this study. Dried samples were crushed using a mortar and a pestle. Approximately 20 mg of crushed sample was weighed out and suspended in 12 mL of 2% nitric acid. Next, the sample was sonicated for an hour to ensure the disaggregating of the spores a fairly consistent suspension. The suspension was then centrifuged for 9 minutes in order to reduce the number and average size of spores in suspension. The supernatant was then separated and was ready for analysis.

Analysis of bacteria samples. Six replicates of each bacteria sample were prepared in parallel. All elements in the sample present above detection limits were measured and identified during a mass scan prior to collecting the elemental ratios to be used for chemical fingerprinting. The elements to be used for fingerprinting were selected based on signal strength and possible polyatomic interferences. The peak spectra for a typical mass scan are presented in figure 1. Elements, which provided the most significant relative differences between samples, were selected for the fingerprint. Finally, the elements selected were normalized to barium since the relative concentration of Ba was similar in the three samples analyzed.

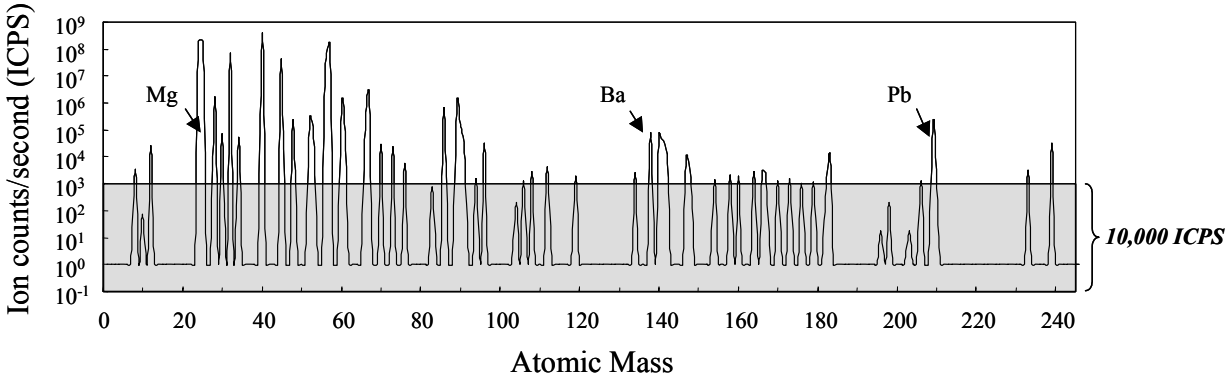

Figure 1. Log-linear plot of ICPS vs. Atomic Mass from a scan of a BG suspension. Detections below 10,000 ICPS were eliminated as possible fingerprint markers.

RESULTS AND DISCUSSION

The mass scans from each sample BG, Bg and Bt indicate that the elements Mg, P, V, Mn, Co, Cu, Zn, Sr, Ba and Pb are present above detection limits (Figure 1). The mean (N=6) concentration (ug/g) for each element selected as a fingerprint component is presented in table 2 for all three bacteria. The results indicate significant relative differences of each element from these samples (table 2).

Table 2. Average elemental composition (ug/g) of the three test organisms (BG, Bg, Bt).

Sample	Element (ug/g)	Mg	P	V	Mn	Co	Cu	Zn	Sr	Ba	Pb
BG spores	*Mean (n=6)*	6593.50	4641.33	6.76	170.13	22.95	26.74	401.65	20.91	10.07	6.84
	SD	384.66	273.85	0.51	10.15	1.36	1.87	18.81	1.26	0.62	0.49
	%RSD	5.83	5.90	7.57	5.96	5.91	7.01	4.68	6.02	6.13	7.18
Bt	*Mean*	1899.33	869.00	0.12	101.08	0.08	12.27	42.61	18.65	16.63	0.33
	SD	109.76	66.98	0.01	2.31	0.01	0.88	9.74	1.06	0.81	0.05
	%RSD	5.78	7.71	6.68	2.28	6.74	7.15	22.85	5.70	4.87	15.63
Bg vegetative cells	*Mean*	6999.83	5643.17	7.16	4342.50	0.52	34.63	452.52	179.43	27.94	30.26
	SD	360.83	302.70	0.32	181.70	0.02	1.47	17.85	6.71	1.03	1.48
	%RSD	5.15	5.36	4.40	4.18	4.38	4.23	3.95	3.74	3.70	4.88

Elemental ratio signatures from each organism were calculated and compared in figure 2. Significant differences between each of the organisms are apparent (Figure 2). The relative differences in the ratios are outside the uncertainty on the measurements indicating that the different chemical fingerprints are valid.

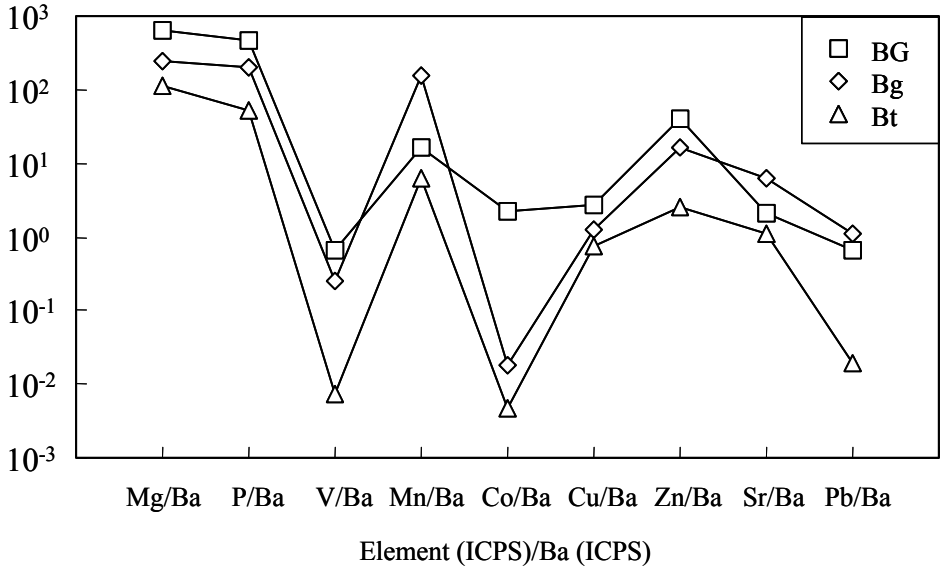

Figure 2. Unique chemical fingerprints for each organism analyzed (BG, Bg, Bt). Error bars are smaller than the symbols plotted.

There are two likely sources for the distinct chemical signatures detected. The first source of variability is likely the result of the methods used to culture the organism. This difference is best demonstrated by comparing the signatures of Bg and BG samples (Figure 2). These organisms would have been cultured and processed using slightly different conditions in order to induce spores versus vegetative cells. A developmental effect may also contribute to the total difference between these samples. The second source of variability may be a species effect. This effect is best observed when comparing the BG to Bt samples (Figure 2). Since these organisms will likely be cultured and processed in similar ways in order to produce spores, the net difference can most likely be attributed to biologic fractionation. These possibilities need to be investigated in more detail; however, our ability to resolve these subtle differences demonstrates the precision of the method described above.

CONCLUSIONS

The results presented here suggest that detection and identification of aerosolized biological materials using an inorganic chemical fingerprint may be a viable method for point detection of biological warfare agents. The development of a unique fingerprint is not limited to the elements selected for this study. The flexibility of using most elements on the periodic table as a means to detect the presence of a bio-warfare agent and potentially distinguish the type of organism, its state (i.e. vegetative vs. spore), and how and/or where it was processed makes for a powerful and precise detection system. In addition, by cataloging the inorganic signature of different species and different preparation methods, a database could be developed that would simplify the interpretation of results making the inorganic fingerprint method useful to untrained personnel. While more work is obviously needed to refine the source of chemical

variability detected in the three samples analyzed, this work represents a major step toward expanding the technologies potentially available as detection systems in the war on terrorism and homeland security.

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